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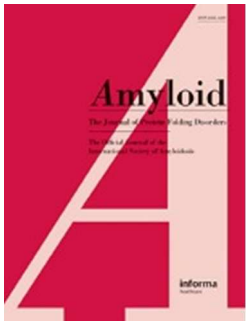

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Extended characterization of the novel co-isogenic C57BL/6J *Prnp*$^{-/-}$ mouse line

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Background. Besides its involvement in peripheral myelin maintenance, the physiologic function of the ubiquitously expressed cellular prion protein PrP^{C} remains enigmatic. Phenotypic studies on previously available *Prnp*$^{-/-}$ mouse lines, produced in 129-derived embryonic stem cells and backcrossed to non-129 strains, were systematically confounded by *Prnp*-linked loci polymorphic between 129 and the backcrossing strain and have led to erroneous conclusions [1]. We have recently applied genome-editing to generate a novel co-isogenic *Prnp*$^{-/-}$ mouse line (termed ZH3 line) in the well characterized C57BL/6J background [2]. Genetic and phenotypic characterization of this line excluded the presence of artefactual phenotypes reported in non-co-isogenic *Prnp*$^{-/-}$ mouse lines and confirmed the crucial involvement of PrP^{C} in peripheral myelin maintenance through the interaction with Gpr126 [2, 3]. Here, we aimed at extending the characterization of the *Prnp*$^{ZH3/ZH3}$ mouse line.

Materials and methods. Data on single nucleotide polymorphism (SNPs) informative among different C57BL/6 substrains were extrapolated from a whole-genome SNP analysis performed as previously described [2]. Genotyping protocols to investigate the presence of mutations/deletions in *Crb1*, *Snca*/*Mmrn1* and *Nnt* were as previously described [4, 5, 6]. Prion inoculations and relative analyses were all performed as previously described [7].

Results. Since its establishment, the C57BL/6 inbred strain has led to the origin of several substrains, which differ genetically and phenotypically with respect to the reference C57BL/6J [8]. To verify the genetic makeup of the *Prnp*$^{ZH3/ZH3}$ mouse line, we analyzed 12 SNPs known to be informative among different C57BL/6 substrains. The *Prnp*$^{ZH3/ZH3}$ line showed an allelotype identical to the C57BL/6J reference strain (Figure 1A). We next investigated the *Prnp*$^{ZH3/ZH3}$ line for the presence of specific deletions or mutations reported in different C57BL/6 substrains using established genotyping assays. The *Prnp*$^{ZH3/ZH3}$ line showed the presence of the partial deletion of *Nnt*, described in the C57BL/6J reference strain [6], but not of the Rd8 mutation of the *Crb1* gene, described in the C57BL/6N strain [4], nor the deletion of *Snca*/*Mmrn1*, reported in the C57BL/6JOlaHsd strain [5] (Figure 1A). Collectively, these data confirmed that the *Prnp*$^{ZH3/ZH3}$ line is indistinguishable with respect to the C57BL/6J reference strain in all investigated genomic loci.
Expression of membrane-bound PrP^{C} is necessary to sustain the replication and neurotoxicity of prions and mice devoid of PrP^{C} resist prion infection. To verify prion resistance of the novel knockout line, we intracerebrally inoculated *Prnp*$^{ZH3/ZH3}$ and C57BL/6J wild-type mice with the 22L prion strain or with non-infectious brain homogenate (NBH) as control. Only 22L-inoculated C57BL/6J wild-type mice developed typical signs of scrapie and achieved the terminal stage of the disease with a median incubation of 141 days post-injection. Conversely, NBH-injected C57BL/6J wild-type mice and both 22L- and NBH-injected *Prnp*$^{ZH3/ZH3}$ mice showed no clinical signs of scrapie and survived more than 200 days (Figure 1B). Histological

analysis confirmed the presence of spongiosis and accumulation of the partially-protease resistant prion protein, typical hallmarks of prion disease, only in 22L-inoculated C57BL/6J wild-type mice and not in any other experimental group. Altogether, these data confirmed the prion resistance of the *Prnp*$^{ZH3/ZH3}$ mouse line.

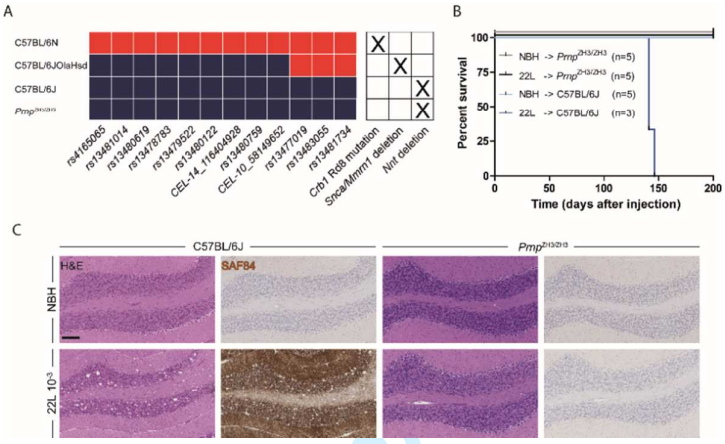

Figure 1. A. SNP and mutation analysis of *Prnp*$^{ZH3/ZH3}$ mice and reference C57BL/6 substrains. X denotes presence of a specific mutation/deletion. **B.** Survival after injection with prions (22L) or control (NBH). n denotes the number of mice in each group. **C.** Histologic analysis of cerebelli of representative mice from B. SAF84 shows partially protease resistant prion protein. Scale bar: 100 µm.

Discussion and conclusions. This study confirms the co-isogenicity of the *Prnp*$^{ZH3/ZH3}$ line with the C57BL/6J reference strain and its resistance to prion infection. Together with the extensive genomic, transcriptomic and phenotypic characterization reported [2, 3], this study corroborates the notion the *Prnp*$^{ZH3/ZH3}$ line is a rigorous genetic resource for investigating the role of PrP^{C} in physiology and disease.

Declaration of interest. The authors have no conflict of interest to report.

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